

Tick-bite-induced anaphylaxis in Spain

BY P. FERNÁNDEZ-SOTO

Departamento de Parasitología, Facultad de Farmacia,

Universidad de Salamanca, 37007 Salamanca, Spain

I. DÁVILA, E. LAFFOND, F. LORENTE

Servicio de Alergología, Hospital Universitario, 37007 Salamanca, Spain

A. ENCINAS-GRANDES

Departamento de Parasitología, Facultad de Farmacia,

Universidad de Salamanca, 37007 Salamanca, Spain

AND R. PÉREZ-SÁNCHEZ*

Departamento de Patología Animal, Instituto de Recursos Naturales y Agrobiología,

CSIC, Cordel de Merinas, 40-52, 37008 Salamanca, Spain

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LH: FERNÁNDEZ-SOTO *ET AL.*

*Author to whom correspondence should be addressed. E-mail: ricarpe@gugu.usal.es; fax: +34 923 219609.

Although there are very few reports of human anaphylaxis induced by tick bites, two such cases have recently been seen in Salamanca, Spain. To identify the tick species responsible, salivary-gland extracts from six species of hard tick and two of soft tick were prepared and used as allergens/antigens in skin-prick tests and serological analyses. For each case, the results of the skin tests were positive for several species of hard tick but negative for the soft ticks. ELISA and western blots revealed high titres of IgG against hard ticks (but not soft ticks) in the sera from both cases. However, serum from only one of the cases was found to be ELISA- and western-blot-positive for tick-specific IgE. Accordingly, the anaphylaxis seen in one case was IgE-mediated whereas that in the other case appeared to be IgE-independent. In both cases, most of the tick-specific antibodies only recognized carbohydrate epitopes. High levels of cross-reactivity between the salivary-gland extracts from several species of hard tick made it impossible to identify which species was responsible for each anaphylactic reaction, although the immunological results seem to point to *Ixodes ricinus*.

Tick bites can lead to human illness by transmitting infectious agents or by introducing toxins capable of causing local irritation, paralysis or even hypersensitivity reactions (Pearn, 1977). There have been few reports of type-I hypersensitivity reactions induced by ticks and most of these have been described either in Australia, where they were caused by *Ixodes holocyclus* (Gauci *et al.*, 1989a; Brown and Hamilton, 1998), or in the U.S.A., where they were induced by *I. pacificus* (Van-Wye *et al.*, 1991b). Two human cases of anaphylactic shock following tick bites in Spain have now been investigated. The results of cutaneous and serological tests on both cases, carried out to try to identify the tick species responsible and the nature of the allergens involved (peptide v. saccharide), are presented here.

PATIENTS AND METHODS

In the summer of 1998, two patients were referred to the Department of Allergy of the University Hospital in Salamanca, Spain, each following an episode of anaphylactic shock. Patient 1 (P1) was a 61-year-old man who worked as a farmer and cattle raiser in a rural area in western Spain. He had undergone an episode of generalized urticaria, angioedema of the lips, eyes, tongue and larynx, severe bronchospasm, hypotension and cyanosis. These signs and symptoms initially responded to intensive treatment with oxygen, epinephrine, antihistamines, corticosteroids and fluid replacement, but recurred 6 h later and were finally controlled with corticosteroids. In the emergency room, a tick was removed from this patient's left testis.

Patient 2 (P2) was a 62-year-old man who also worked as a farmer and cattle raiser and was from a region of western Spain close to the home of P1. He had developed generalized urticaria, facial and laryngeal angioedema, loss of consciousness, hypoxemia and acidosis. These signs and symptoms were successfully treated with intensive treatment in the emergency room (oxygen, epinephrine, corticosteroids, antihistamines and intravenous fluids) and did not then recur. A tick was removed from the genitalia of P2. On recovery, P2 reported that he had had a similar response to a tick bite 20 years previously.

Unfortunately, the ticks removed from P1 and P2 were discarded before being identified to species. The possible association of the observed anaphylaxis with tick feeding therefore had to be investigated indirectly, using skin-prick tests and serological investigation of sera isolated from blood samples collected 32 days (P1) or 13 days (P2) after each patient was bitten by the tick recovered subsequently.

To rule out the possible causes of anaphylactic shock other than tick bite, skin-prick tests to a common battery of pneumoallergens and foods were performed and serum titres of specific IgE to *Echinococcus granulosus*, *Ascaris lumbricoides* and *Anisakis simplex* were assessed. The total serum concentrations of IgE were also estimated by routine methods (Radioimmunoassay).

In an attempt to identify the tick species responsible for the anaphylactic shock in each of the two patients, extracts were prepared (Pérez-Sánchez *et al.*, 1992) from salivary glands dissected out of the six

species of hard tick (*Rhipicephalus bursa*, *R. sanguineus*, *R. turanicus*, *Hyalomma marginatum*, *Dermacentor marginatus*, *Ixodes ricinus*) that are common in the area where the patients live. Similar extracts were also prepared from two species of soft tick (*Ornithodoros moubata* and *O. erraticus*) known to be vectors of human pathogens, although these two species are not present in the area where the patients live. For each extract, 100 pairs of salivary glands of one species were dissected out, washed and suspended in 2 ml 10 mM phosphate-buffered saline (PBS) at pH 7.2. The suspension was then frozen and thawed five times and the resulting supernatant solution was removed, filtered through a membrane filter with 0.2- μ m pores and subsequently used as a salivary-gland extract (SGE). The protein concentration of each extract was estimated by the method of Bradford (1976).

The SGE, either in their native form (SGE-N) or after being deglycosylated (SGE-D), were used as antigens in ELISA and western blots, to detect serum titres of anti-tick IgG and IgE antibodies. Deglycosylation was accomplished with sodium metaperiodate (Woodward *et al.*, 1985; Oleaga-Pérez *et al.*, 1994) after the SGE had been fixed to microtitre plates (1 μ g/well) or subjected to SDS-PAGE (in 5%–20%, gradient mini-gels, at 15 μ g/lane) and electroblotted onto nitrocellulose sheets. The plates or sheets were washed with 50 mM acetate buffer (pH 4.5) for 2 min at room temperature, incubated with 10 mM sodium metaperiodate in the same buffer for 2 h at 4°C, washed in PBS, and post-incubated in 1% glycine in PBS for 1 h, again at 4°C. After washing again in PBS, the plates and sheets were ready for serology: ELISA and western blots, respectively.

The ELISA with native and deglycosylated SGE were run in parallel, following the protocol described by Canals *et al.* (1990) with some modifications. The plates, either coated with SGE-N or with SGE-D, were post-coated with 1% bovine serum albumin (BSA) and incubated with a test serum at two different dilutions: 1:50 to search for IgG antibodies and 1:10 to look for IgE antibodies. Then, they were re-incubated with the relevant, peroxidase-labelled conjugate (each from Sigma): anti-human IgG (diluted 1:4000) or anti-human IgE (diluted 1:500). Finally, the positive reactions were revealed using O-phenylenediamine as chromogenic substrate.

The western blots with native and deglycosylated SGE were also carried out in parallel (Pérez-Sánchez *et al.*, 1992; Oleaga-Pérez *et al.*, 1994). Briefly, the nitrocellulose sheets were post-coated in 1% BSA, incubated with a test serum diluted 1:50 dilution (for IgG) or 1:10 dilution (for IgE) and re-incubated, as appropriate, with peroxidase-labelled anti-human IgG (1:1000) or anti-human IgE (1:500). The reactive bands were stained using 4-chloro-1-naphthol as chromogen.

Both patients and 10 apparently healthy subjects (used as controls) were subjected to skin-prick tests (Dreborg and Frew, 1993) in which the allergens used were SGE-N which had been irradiated with ultraviolet light for 90 min.

RESULTS

Total serum concentrations of IgE were 51 ng/ml in P1 and 180 ng/ml in P2, both inside the normal range (0–300 ng/ml) for non-atopic individuals. The sera from the patients showed no reactivity with SGE from species of soft tick but did react with some extracts from species of hard tick (see below).

ELISA

IMMUNOGLOBULIN G

In the ELISA for IgG against the SGE-N of hard ticks (Fig. 1), the optical densities (OD) obtained with the sera of both patients were several-fold higher than those of the negative control sera (0.046–0.076). For each patient, the highest OD seen was produced when the *I. ricinus* SGE-N was used as antigen. When the SGE-D were used as the antigens, the OD seen with the test sera were always the same or only marginally higher than those seen with the control sera.

[f]Figure 1 here[/f]

IMMUNOGLOBULIN E

In the ELISA for IgE (Fig. 1), the serum from P2 did not react with any SGE, native or deglycosylated, whereas that of P1 reacted with SGE-N from several species of hard tick (mainly with those of *I. ricinus* and *D. marginatus*) but not with any SGE-D.

Western Blots

IMMUNOGLOBULIN G

In western blots for IgG, the sera from both patients revealed numerous bands when hard-tick SGE-N were used as the antigens but no bands when any SGE-D were used. As IgG banding patterns are of little value in the investigation of anaphylaxis, the IgG blots are not shown.

IMMUNOGLOBULIN E

In the western blots for IgE, the P1 serum produced some banding when the antigen was the SGE-N from each of several hard-tick species (Fig. 2). The most intense bands were seen when *D. marginatus* SGE-N was the antigen, with six bands between 17 and 38 kDa. Numerous (although less intense) bands, forming two smears between 18 and 106 kDa, could also be seen when *R. sanguineus* SGE-N or *I. ricinus* SGE-N was the antigen source. Only a few faint bands, if any, could be seen when the other SGE-N were used, and no banding could be detected with any SGE-D.

[f]Figure 2 here[/f]

Skin-prick Tests

Neither patient reacted to any of the non-tick allergens used in the skin-prick tests. None of the controls showed any reaction to any tick allergen inoculated in the cutaneous tests. However, P1 reacted to the SGE-N of *R. bursa*, *R. sanguineus* and *I. ricinus* and P2 reacted to the SGE-N of *D. marginatus*, *R. bursa*, *R. sanguineus* and *I. ricinus*.

DISCUSSION

The recovery of a tick from each of the two patients investigated in the present study indicated that tick bites might be the cause of the anaphylaxis with which the men presented. Once most of the possible, non-tick causes of anaphylactic shock had been ruled out by the results of skin-prick tests, the possible role of tick salivary antigens in the anaphylaxis was investigated. The results of the skin tests performed with these antigens showed that both patients were in fact sensitized to the antigens from several species of hard tick, making it difficult to identify which (if any) of the tick species investigated caused the anaphylaxis. The results of the serology confirmed that both patients had been exposed to tick salivary antigens, since both were carriers of anti-tick serum IgG. Furthermore, the results for P1 —indicating the presence of anti-tick IgE in his serum and a late-phase reaction— clearly indicated that his anaphylaxis, like most cases of anaphylactic shock (MacDonald *et al.*, 1995), was IgE-mediated.

It was surprising to find that the other patient, P2, apparently lacked anti-tick IgE antibodies, although this result does not mean that his anaphylaxis could not have been IgE-mediated. The titres of tick-specific IgE in his serum may simply have been too low to detect in the indirect ELISA employed. When P2 was bled for the present study, the bulk of his anti-tick IgE antibodies might have been bound to mast cells and basophils, as the result of prior exposure to the tick species involved months or years previously; the anaphylactic reaction suffered by P2 some 20 years prior to the present study and the positive skin reactions to some tick salivary antigens support this idea. The between-patient difference in the number of days between tick bite and serum collection may also account for the difference in tick-specific IgE titres, P2 perhaps having been bled 'too soon' (i.e. before his IgE response had reached a detectable level). There is, however, increasing evidence that anaphylactic reactions may occur in the total absence of IgE (Marquardt and Wasserman, 1993; Oettgen *et al.*, 1994; Corry and Kheradmand, 1999) and that IgG antibodies can generate hypersensitivity reactions (Colten, 1994; Oettgen *et al.*, 1994; Bergamaschini *et al.*, 1995; Bozic *et al.*, 1996; Corry and Kheradmand, 1999). P2's anaphylaxis may therefore have been IgG-mediated, although the present results are insufficient to prove or disprove this idea.

That neither of the sera from the patients reacted with any deglycosylated SGE indicates that, in both patients, most of the anti-tick antibodies recognized carbohydrate epitopes. However, it cannot be concluded that carbohydrate epitopes (at least in P1) were responsible for the anaphylactic reactions because the antigens from *R. bursa* were the most reactive in the skin-prick tests even though the results of subsequent studies indicate that these antigens were the least glycosylated of those investigated (unpubl. obs.)

The strong cross-reactivity seen between the salivary antigens of the species of hard tick investigated in the present study (but not between these and those of the soft ticks also included; Figs. 1 and 2) does not permit the species responsible for the anaphylactic reactions to be identified. Cross-reactions between hard-tick salivary antigens have been reported several times previously (Pérez-Sánchez *et al.*, 1992). To date, all known cases of anaphylaxis caused by the bites of hard tick have been ascribed to species of the genus *Ixodes* (Pearn, 1977; Gauci *et al.*, 1989a, b, c; Van Wye *et al.*, 1991a, b; Moneret-Vautrin *et al.*, 1996; Brown and Hamilton, 1998). In the present study, the antigens reacting most strongly with the sera from P1 and P2 came from *I. ricinus*. This species is therefore considered the most likely cause of the anaphylactic reactions, especially as residents of the area in which P1 and P2 live are frequently bitten by this species (Fernández-Soto *et al.*, 1997). It would be interesting to see if some allergenic epitope associated with the genus *Ixodes* exists. If such an epitope does not

exist, then any species could have acted as a sensitizer and any other species could have been responsible for triggering the anaphylactic reaction.

In conclusion, ticks are able to induce life-threatening, IgE-mediated anaphylactic reactions in humans. The possibility of a tick bite should therefore be investigated in cases of anaphylactic shock with no other apparent cause.

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Fig. 1. ELISA indices (i.e. optical densities for patient sera divided by the mean optical densities for the negative-control sera) recorded for patients 1 and 2. The antigens used were native (SGE-N) or deglycosylated (SGE-D) extracts of the salivary glands of *Dermacentor marginatus* (Dm), *Rhipicephalus bursa* (Rb), *R. turanicus* (Rt), *R. sanguineus* (Rs), *Ixodes ricinus* (Ir), *Hyalomma marginatum* (Hm), *Ornithodoros erraticus* (Oe) or *O. moubata* (Om). Some of the native extracts (*) produced positive results when used in skin-prick tests.

Fig. 2. Western blots showing the tick antigens recognized by the serum IgE of patient 1. The antigens used were native extracts (upper panel) or deglycosylated extracts (lower panel) of the salivary glands of *Dermacentor marginatus* (Dm), *Rhipicephalus bursa* (Rb), *R. turanicus* (Rt), *R. sanguineus* (Rs), *Ixodes ricinus* (Ir), *Hyalomma marginatum* (Hm), *Ornithodoros erraticus* (Oe) or *O. moubata* (Om). Cross-reactivity between the native salivary antigens of several species of hard tick can be observed.